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Analytic Sensitivities of Hybrid-Capture, Consensus and Type-Specific Polymerase Chain Reactions for the Detection of Human Papillomavirus Type 16 DNA

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Human papillomavirus type 16 (HPV-16) DNA is detected commonly in cervical carcinomas; in this study, we have determined the analytical sensitivities of Hybrid Capture, HPV-consensus PCR, and three HPV-16-specific polymerase chain reactions (PCRs) for the detection of HPV-16 DNA. Samples investigated included a cervical cancer cell line, cervical scrapes from 20 patients attending colposcopy clinics, and buccal swabs from eight immunosuppressed children. HPV-16 E7 and E5-nested PCRs [Cavuslu et al. (1996): Journal of Virological Methods, in press] produced positive signals from samples containing fewer than ten HPV-16 genomes per reaction. HPV-consensus PCR [Manos et al. (1989): Cancer Cells 7:209–214] and HPV-16 PCR using primers of van den Brule et al. [(1990): Journal of Clinical Microbiology 25:2739–2743] were of intermediate sensitivity (i.e., produced positive signals from samples containing 250 and 2,500 HPV-16 genoms/reaction, respectively) and Hybrid Capture could detect just 50,000 HPV-16 genomes/reaction. Highest rates of positivity for cervical samples were detected with HPV-16 E7 or E5-nested PCRs [50% (10 of 20 samples) and 60% (12 of 20 samples) positive, respectively], intermediate rates with HPV-consensus PCR and PCRs using the primers of van den Brule et al. [both 35% (7 of 20 samples)], and lowest rates of positivity [25% (5 of 20 samples)] with Hybrid Capture. None of eight buccal swab samples from immunosuppressed children were positive by Hybrid Capture, yet three (37.5%) were positive by HPV-16 E5-nested PCR. These data indicate that HPV-16 type-specific PCRs should be used for the investigation of specimens that may contain low amounts of HPV-16 DNA.

KEY WORDS: human papillomavirus type 16 DNA, hybrid capture, PCR

INTRODUCTION

More than 70 human papillomavirus (HPV) genotypes have been identified, of which about 30 are mucosatropic [de Villiers, 1992]. Among mucosal HPVs, infections with type 16 or type 18 (and also, but less frequently, types 31, 33, 35, 45, 51, 52, and 56) are considered to be high risk; these types are strongly associated with cervical carcinoma and its precursor lesion, cervical intraepithelial neoplasia (CIN) [zur Hausen, 1989, 1994; Syrjanen, 1989].

Cytological, histological, and electron microscopic studies can provide evidence of HPV infection but do not permit the identification of HPV types. Owing to difficulties in propagating HPVs, there are as yet no entirely reliable specific serological tests for different HPVs. Thus, detection of HPV genotypes depends on the use of 1) HPV type-specific nucleic acid probes in methods such as in situ hybridization, dot blotting, Southern blotting, and filter in situ hybridization (FISH); 2) amplification of viral DNA by the polymerase chain reaction (PCR) using HPV-specific or consensus primers; and 3) the Hybrid (DNA-RNA) Capture assay [O'Banion et al., 1987; Caussy et al., 1988; Munoz et al., 1988; Manos et al., 1989; van den Brule et al., 1990].

We are interested in high-risk HPV infections among infants and children. In such instances, HPV-DNA may be present but at low copy number [Fredericks et al., 1993; Pakarian et al., 1994; Kaye et al., 1994, 1996; Cason et al., 1995b]. Hybrid Capture potentially offers

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several advantages over HPV type-specific PCRs for such investigations, namely, the ability to detect a multiplicity of high-risk HPV types and a standardised format with an objective, quantitative endpoint. We have used previously a policy of screening for HPV infection using a consensus PCR assay and then typing positive cases using HPV-specific PCRs [Pakarian et al., 1994]. The validity of this approach presumes that the analytical sensitivities of the consensus and type-specific PCRs are similar. However, often, this is not the case, necessitating reassay of samples with type-specific PCRs in order to secure accurate data [Cason et al., 1995a].

To determine whether Hybrid Capture would be of use for studies of HPV-16 infections in children, we have compared the analytical sensitivities of this test and of an HPV-consensus PCR with those of three PCRs using HPV-16 type-specific primers. Samples investigated included an HPV-16-containing cervical cancer cell line: cervical scrapes from 20 women attending colposcopy clinics, nine of whom had cytological evidence of low-grade HPV infections and one a high-grade CIN lesion; and buccal swabs from eight immunosuppressed teenage renal allograft recipients.

MATERIALS AND METHODS

Cervical Carcinoma Cell Line

SiHa cells were obtained from the American Type Culture collection (ATCC; catalogue No. HTB-35): these contain one HPV-16 genome per cell [El Awady et al., 1987]. Cells were maintained in culture, counted, pelleted by centrifugation, and prepared for analyses (below).

Clinical Samples

Cervical scrapes were collected from 20 women (mean age 33 years, range 20–57 years) attending colposcopy clinics at St. Thomas' Hospital. Seven women had CIN (six with CIN-I and one CIN III), and three had cervical cytology indicative of HPV (wart virus changes); ten women had normal cervical cytology results. In addition, we collected buccal swabs from eight teenagers (mean age 14 years, range 12–18 years) who had received renal transplants between 1 and 10 years previously and were receiving prednisolone, cyclosporin A, and azathioprine. Collection of these samples was approved by the research ethics committees of Guy's and of St. Thomas' Hospital, London.

Sample Preparation

Samples were collected into 1 ml of sterile distilled water, mixed, and then divided into aliquots (0.5 ml for PCRs and 0.5 ml for Hybrid Capture). Samples for Hybrid Capture were pelleted by centrifugation and resuspended in 0.5 ml of transport medium (Digene Diagnostics, Inc.); aliquots for PCR were treated with proteinase K [Kaye et al., 1994].

Hybrid Capture Assay

Hybrid Capture assays (Digene Diagnostics, Inc.) were performed according to the manufacturer's instructions to detect high-risk HPV DNA in 100 μl aliquots of clinical samples using probe B (which contains a cocktail of RNA probes for HPV-16, -18, -31, -33, -35, -45, -51, -52, -56). Chemiluminescence was quantified in a luminometer (DCR-1 Luminometer: Digene Diagnostics, Inc.) as relative light units (RLU). Positivity for high-risk HPVs was defined as an RLU value in excess of the mean RLU of three positive controls (HPV-16 DNA) included in the kit.

HPV-Consensus PCR

Consensus PCR was carried out using degenerate HPV-consensus primers MY09/MY11, which can amplify DNA from the vast majority of genital HPVs [Manos et al., 1989], using PCR cycling conditions and magnesium concentrations optimised as described previously [Cavuslu et al., 1996].

PCRs for HPV-16, -18, -31, -33 DNA

PCRs for these HPVs can be performed in a single "multiplex" PCR to produce amplicons of different molecular sizes according to the type of HPV present [van den Brule et al., 1990]. However, in this study, each pair of HPV type-specific primers was used in individual PCRs employing optimised magnesium concentrations and cycle conditions.

PCRs for HPV-16 DNA Using E5-Nested and E7 Primers

These PCRs were carried out using conditions described previously and are specific for HPV-16 DNA [Cavuslu et al., 1996].

β-Globin PCRs

Samples were assayed by PCR using β-globin primers PC03 and PCO4 [Saiki et al., 1986] to confirm that all contained sufficient DNA for HPV analyses and that nonspecific inhibitors of PCRs were absent.

All PCRs were undertaken under conditions designed to minimise contamination [Muir et al., 1993]. Each PCR run included two negative controls (A431 cells and water) and one positive control (pAt/HPV-16 DNA; from Dr E.M. DeVilliers, DKFZ, Heidelberg, Germany) per eight clinical samples tested. After amplification, 15 μl of each PCR reaction was analysed via agarose gel electrophresis.

Statistical Tests

Student's unpaired t tests were used in the interpretation of data.

RESULTS

Hybrid Capture Assay Validation

Mean RLU for negative controls by Hybrid Capture was 4,877 RLU [coefficient of variation (cv) = 4.8] and for positive controls 15,908 RLU (cv = 4.9%). The assay thus fell within the manufacturer's recommendations (ratio between RLU for positive and negative controls), and a cutoff value of 15,908 RLU was used to define positivity.

TABLE I. Analytical Sensitivities of Assays for HPV-16 DNA Detection*

Copies	H. cap.	Polymerase chain reactions			
		vdB	MY09/11	16-E7	16-E5n
50,000	+	+	+	+	+
25,000	−	+	+	+	+
2,500	−	+	+	+	+
250	−	−	+	+	+
25	−	−	−	+	+
2.5	−	−	−	+	+

*Copies, number of SiHa cell-derived HPV-16 genomes per reaction tube [copy number was deduced from the number of SiHa cells; each cell contains 1 copy of HPV-16 genome (El Awady *et al.*, 1987)]; +, positive in that assay: −, HPV not detected. H. cap., Hybrid Capture assay for high-risk HPVs; MY09/11, PCR using MY09/11 HPV consensus primers; vdB, result of PCRs using primers specific for HPV-16 as described by van den Brule *et al.* [1990]; 16-E7, PCR for HPV-16 using E7 primers; 16-E5n, nested PCR for HPV-16 DNA using E5 primers.

Analytic Sensitivity for the Detection of HPV-16 DNA

Analyses of serial dilutions of SiHa-cell HPV-16 DNA reveal that Hybrid Capture had a sensitivity limit of 50,000 HPV-16 genomes/reaction, whereas all PCRs were more sensitive (Table I). PCRs using HPV-16-specific primers of van den Brule et al. [1990] were able to reveal as few as 2,500 genomes/reaction; MY09/11 HPV-consensus primers as few as 250 genomes/reaction, and HPV-16 E5-nested or HPV-16 E7 primers as few as 2.5 genomes/reaction.

Cervical Scrapes

All 20 samples were positive in PCRs for β-globin amplicons (Table II). Overall, five of 20 (25%) samples were positive by Hybrid Capture for high-risk HPVs, whereas seven (35%) were positive for HPV-DNA in PCRs using MY09/11 primers. In PCRs using HPV-specific primers of van den Brule et al. (1990), seven (35%) samples were positive (three contained HPV-16; two had HPV-31, and two others had HPV-33 DNA). By HPV-16 E7 PCR, ten (50%) samples were positive, and by nested E5-PCR 12 (65%) samples were positive. Samples from five women (25%; cases 1, 7, 8, 13, 27, and 28), all of whom had normal cervical cytology results, were negative for HPV DNA in all assays.

Among five samples positive for high-risk HPVs by Hybrid Capture, four (80%) were positive in PCRs using MY09/11 primers, and all five (100%) were positive in PCRs using HPV type-specific primers of van den Brule et al. [1990]: Two were positive for HPV-16 DNA, one for HPV-33, and another for HPV-31. Both samples that were positive by Hybrid Capture (cases 5 and 26) and for HPV-16 DNA by PCR using the van den Brule et al. [1990] primers were also positive for HPV-16 in PCRs using E5-nested and E7 primers. One sample (case 24) that was positive by Hybrid Capture and by PCR using the van den Brule et al. [1990] primers for HPV-33 DNA was also positive by HPV-16 E5-nested PCR. In no case was a sample positive by PCR for HPV-16 DNA using HPV-16 E7 or van den Brule primers and negative by PCR using HPV-16 E5-nested primers, although, conversely, three samples (cases 4, 14, and 24) were negative by HPV-16 E7 PCR yet positive in HPV-16 E5-nested PCR.

Dilutions of HPV-16 DNA below the cutoff value of 15,908 RLU produced a dose-response curve (data not shown), therefore, the relationship between RLU values for samples determined to be Hybrid Capture negative but PCR positive was investigated. The mean RLU values for samples that were PCR positive exceeded mean RLU values for PCR-negative samples for all PCRs; these differences were not significant ($P > 0.05$; Table III).

Diagnostic Sensitivity of Assays To Detect HPV-Induced Pathology

Diagnostic sensitivity for CIN ranged from 42.8% (three of seven samples) positivity for the HPV-16 E7 primer-based PCR, through 57.1% (four of seven samples) for Hybrid Capture, to 71.4% (five of seven samples) for the three remaining assays (Table IV). Only PCRs using HPV-16 E5-nested and E7 primers were able to reveal HPV DNA in three of three (100%) and two of three (66.6%) samples, respectively, from patients with wart virus changes. When the data for samples from women with wart virus changes or CIN were combined, positivity rates ranged from 40% (four of ten patients) when Hybrid Capture was used, through 50% [five of ten samples; in PCRs using the MY09/11, van den Brule et al. (1990), HPV-16 E7 primers], to 80% (eight of ten samples) for the PCR using HPV-16 E5-nested primers. Positivity among samples from women with normal cervices ranged from 10% (one of ten samples) for Hybrid Capture, through 20% [two samples; for PCRs using MY09/11 and van den Brule et al. (1990) primers], to 50% (five of ten samples) for HPV-16 E5-nested or E7 PCR.

Buccal Swabs From Teenagers

Hybrid Capture and E5-nested PCR were also used to study buccal swabs from children who were immunosuppressed. None of the eight samples was positive by Hybrid Capture, whereas three of eight (37.5%) were positive by HPV-16 E5-nested PCR. All eight samples were positive by PCR for β-globin.

DISCUSSION

We determined the analytical sensitivities of five assays for the detection of HPV-16 DNA. The most sensitive tests were PCRs using HPV-16 E7 and E5-nested primers [Cavuslu et al., 1996], which produced positive signals from samples containing 2.5 HPV-16 genomes per PCR reaction. HPV-consensus PCR [Manos et al., 1989] and, HPV-16 type-specific PCRs using the primers of van den Brule et al. [1990] were of intermediate sensitivity. Hybrid Capture was insensitive and could detect only 50,000 HPV-16 genomes/reaction. Sensitivities of the PCRs described herein may be enhanced by the usage of sensitive indicator systems [e.g., using the SHARP signal detection system (Terry et al., 1994) or enzyme-based reactions (Cavuslu et al., 1996)], rather than an

TABLE II. HPV DNA in Cervical Scrapes*

Case	Lesion	H. cap.	Polymerse chain reactions β-Globin	MY9/11	vdB	16-E7	16-E5n
26	CIN-I	+	+	+	16	+	+
24	CIN-III	+	+	+	33	−	+
9	CIN-I	+	+	−	31	−	−
23	CIN-I	+	+	+	31	−	−
2	CIN-I	−	+	+	16	+	+
3	CIN-I	−	+	+	−	+	+
4	CIN-I	−	+	−	−	−	+
7	WVC	−	+	−	−	+	+
6	WVC	−	+	−	−	+	+
14	WVC	−	+	−	−	−	+
5	N	+	+	+	16	+	+
10	N	−	+	+	33	+	+
11	N	−	+	−	−	+	+
25	N	−	+	−	−	+	+
12	N	−	+	−	−	+	+
8	N	−	+	−	−	−	−
13	N	−	+	−	−	−	−
27	N	−	+	−	−	−	−
28	N	−	+	−	−	−	−
1	N	−	+	−	−	−	−
Total		5/20	20/20	7/20	7/20	10/20	13/20
Percentage		25	100	35	35	50	65

*CIN, patients with cervical intraepithelial neoplasia; WVC, cytological changes indicative of wart virus infection; N, patients with normal cevical cytology; HC, Hybrid Capture assay for high-risk HPVs; β-globin, PCR for β-globin DNA; MY09/11, PCR using MY09/11 HPV-consensus primers; vdB, result of four PCRs using primers specific for HPV-16, -18, -31, and -33; 16-E7, PCR for HPV-16 using E7 primers; 16-E5n, nested PCR for HPV-16 DNA using E5 primers; +, DNA detected; −, DNA not detected. Data are summarised as the number of positive samples over the total number tested and as the percentage of samples positive.

TABLE III. Relationship Between RLU Data and PCR Findings for Hybrid Capture Negative Cervical Samples

	PCR results +	n	−	n	*P*
MY09/11	6,266 ± 1,021	3	5,124 ± 715	12	n.s.
vdB	6,572 ± 1,235	2	5,165 ± 700	13	n.s.
16-E7	5,652 ± 924	8	5,011 ± 745	7	n.s.
16-E5n	5,517 ± 865	10	5,025 ± 907	5	n.s.

*Results expressed as the mean RLU ± standard deviation. n, Number of samples in each category; +, Samples considered HPV positive; −, HPV DNA not detected in respective PCR assays; n.s., not significant ($P > 0.5$). MY09/11, PCR using MY09/11 HPV-consensus primers; vdB, result of four PCRs using primers specific for HPV-16, -18, -31, -33; 16-E7, PCR for HPV-16 using E7 primers; 16-E5n, nested PCR for HPV-16 DNA using E5 primer pairs.

TABLE IV. Diagnostic Sensitivities of Assays*

Assay	N (%)	WVC (%)	CIN (%)	WVC or CIN (%)
H. cap.	10 (1/10)	0 (0/3)	57.1 (4/7)	40 (4/10)
MY09/11	20 (2/10)	0 (0/3)	71.4 (5/7)	50 (5/10)
vdB	20 (2/10)	0 (0/3)	71.4 (5/7)	50 (5/10)
16-E7	50 (5/10)	66.6 (2/3)	42.8 (3/7)	50 (5/10)
16-E5	50 (5/10)	100 (3/3)	71.4 (5/7)	80 (8/10)

*N, samples from patients with normal cervical cytology; WVC, samples from patients with cytological changes indicative of wart virus infection; CIN, samples from patients with cervical intraepithelial neoplasia; HC, Hybrid Capture for high-risk HPVs; MY09/11, PCR using MY09/11 HPV-consensus primers; vdB, result of four PCRs using primers specific for HPV-16, -18, -31, -33; 16-E7, PCR for HPV-16 using E7 primers; 16-E5n, nested PCR for HPV-16 DNA using E5 primer pairs. Data are expressed as the percentage of samples positive within each group and, in parentheses, as the number of positive samples over the total number tested.

endpoint of visualisation of amplicons in ethidium bromide-stained gels. Nevertheless, the relative differences between the analytical sensitivities of individual PCRs may remain.

When 20 cervical samples were analysed, the highest rates of HPV-16 DNA detection were observed when PCRs using HPV-16 E7 or E5-nested primers were used (50% and 65%, respectively), intermediate rates with MY09/11 PCR and PCRs using the primers of van den Brule et al. [1990] (both 35%), and lowest rates (25%) with Hybrid Capture. However, it is our experience that, for clinical samples, the sensitivity of HPV-16 E7 PCR may be considerably less than that of E5-nested PCR (Kell et al., unpublished observations). Indeed, in the present study, all samples positive by PCR using HPV-16 E7 primers were also positive by PCR using HPV-16 E5-nested primers. However, the converse was not true, in that three samples were negative by HPV-16 E7 PCR yet positive by E5-nested PCR.

One sample (case 24) was positive by Hybrid Capture and by PCR using MY09/11 primers, and with the van den Brule primers it contained HPV-33 DNA. This sample was negative by HPV-16 E7 PCR yet positive for HPV-16 DNA in the E5-nested PCR. Insofar as HPV-16 E5-nested PCR is HPV-16 type specific [Cavuslu et al., 1996], this case probably represents a dual HPV-16/-33 infection, with low quantities of HPV-16 DNA.

The Hybrid Capture kit contains a positive control (HPV-16 DNA), which acts to provide a cutoff value in RLU to define positivity. To investigate the possibility that RLU values below the cutoff reflect low levels of HPV DNA, we compared RLU values for cervical samples that were negative by Hybrid Capture with respect to PCR findings. In all PCR assays, mean RLU values for PCR-positive samples exceeded that for PCR-negative samples; however, these differences were small and were not statistically significant.

We also investigated buccal swabs from eight iatrogenically immunosuppressed teenagers, four of whom had cutaneous warts. None of the samples from these children was positive for high-risk HPV DNA by Hybrid Capture, but three of eight (37.5%) samples were positive by HPV-16 E5-nested PCR.

Selection of assays appropriate for the detection of an infectious agent may differ according to the type of information required. Clinicians may desire an assay to provide data that are predictive of, or diagnostic for, an infection-related lesion. In contrast, epidemiologists may want information on the number of people infected irrespective of disease. Thus, two criteria for a particular test exist: analytical and diagnostic sensitivity. It was therefore discomforting to note that, whereas Hybrid Capture had a low analytic sensitivity, it also had a low diagnostic sensitivity for patients with low-grade lesions. Only four of seven (57.1%) samples from patients with CIN and none of three from women with cytologically detectable wart virus changes were positive for high-risk HPVs by this technique. In contrast, diagnostic sensitivities of PCRs for HPV associated lesions were higher, up to 80% for the HPV-16 E5-nested PCR. However, in all cases, there was a concomitant increase in the prevalence of detectable HPV among those women with normal cervical cytologies.

Because only a small number of samples from patients (mostly with low-grade HPV-related lesions) were included in this study, it is possible that, for larger numbers of high-grade CIN lesions, the diagnostic sensitivity of Hybrid Capture could be high. However, this seems unlikely; the manufacturers of the Hybrid Capture test appear to (tacitly) acknowledge the shortcomings of the assay; they are about to introduce a microtitre plate-based test (HCM; Digene Diagnostics, Inc.) that has a tenfold increase in analytic sensitivity and a higher diagnostic sensitivity for CIN-II/III lesions than the Hybrid Capture assay [Lorincz, 1996].

Other techniques are available for the detection of HPV-DNA, and all have been found to be relatively insensitive compared to PCRs using consensus primers. Indeed, comparison of HPV prevalences in populations invariably indicates that, when PCRs using HPV consensus (MY09/11) primers or the type-specific primers of van den Brule et al. [1990] are used, prevalence of HPV positivity is substantially lower when the same populations are analysed by Southern-blotting [Schiffman et al., 1991]; Virapap [Gravitt et al., 1990], FISH [Melchers et al., 1989], or dot-blots [Morris et al., 1990]. Indeed, the analytical sensitivities of PCR and dot blots in the latter studies indicate relative sensitivities of 5,000 and 50,000 HPV copies, respectively [Morris et al., 1990].

In conclusion, this study demonstrates that Hybrid Capture assay is inappropriate for the detection of HPV-16 DNA in samples containing low amounts of virus. Accordingly, care should be exercised in 1) interpretation of previous epidemiological investigations based on the use of this technique and 2) selection of this method for future studies concerned with defining the presence of HPV-16 DNA per se as opposed to large quantities of HPV-16 DNA.

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REFERENCES

Cason J, Kaye JN, Best JM (1995a): Non-sexual acquisition of genital human papillomaviruses. Papillomavirus Report 6:1–7.

Cason J, Kaye JN, Jewers RJ, Kambo PK, Bible JM, Kell B, Shergill B, Pakarian F, Raju KS, Best JM (1995): Perinatal infection by, and persistence of, human papillomavirus types 16 and -18 in infants. Journal of Medical Virology 47:209–218.

Caussy D, Orr W, Dean Daya A, Roth P, Reeves W, Rawls W (1988): Evaluation of methods for detecting human papillomavirus deoxyribonucleic sequences in clinical specimens. Journal of Clinical Microbiology 26:236–243.

Cavuslu S, Starkey WG, Kaye JN, Biswas C, Mant C, Kell B, Rice P,

Best JM, Cason J (1996): Detection of human papillomavirus type-16 (HPV-16) DNA utilising microtitre-plate based amplification reactions and a solid-phase enzyme-immunoassay detection system. Journal of Virological Methods (in press).

de Villiers E-M (1992): Laboratory techniques in the investigation of human papillomavirus infection. Genitourinary Medicine 68:50–54.

El Awady MK, Kaplan JB, O'Brian SJ, Burk RD (1987): Molecular analysis of integrated human papillomavirus 16 sequences in the cervical cancer cell line SiHa. Virology 159:389–398.

Fredericks BD, Balkin A, Daniel HW, Schonrock J, Ward B, Frazer IH (1993): Transmission of human papillomavirus from mother to child. Aust NZ Obstet Gynecol 33:30–32.

Gravitt P, Hakenwerth A, Stoerker J (1990): A direct comparison of methods proposed for widespread screening of human papillomavirus infections. Molecular and Cellular Probes 5:65–72.

Kaye JN, Cason J, Pakarian FB, Jewers RJ, Kell B, Bible JM, Raju KS, Best JM (1994): Viral load as a determinant for transmission of human papillomavirus type 16 from mother to child. Journal of Medical Virology 44:415–421.

Lorincz A (1996): Hybrid capture method for detection of human papillomavirus DNA in clinical specimens. Papillomavirus Report 7:1–5.

Manos M, Ting Y, Wright DK, Lewis AJ, Broker TR, Wolinsky SM (1989): Use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. Cancer Cells 7:209–214.

Melchers W, van den Brule A, Walboomers J, de Bruin M, Hebrink P, Meier C, Lindeman J, Uint W (1989): Increased detection rate of human papillomavirus in cervical scrapes by the polymerase chain reaction as compared to modified FISH and Southern blot-analysis. Journal of Medical Virology 27:329–335.

Morris BJ, Rose BR, Flanagan JL, McKinnon J, Loo CY, Thompson CH, Flampoulidou M, Ford RM, Hunter JC, Nightingale BN, Cossart YE (1990): Automated polymerase chain reaction for papillomavirus screening of cervicovaginal lavages: Comparison with dot-blot hybridization in a sexually transmitted diseases population. Journal of Medical Virology 32:22–30.

Muir P, Nicholson F, Jhetam N, Neogi S, Banatvala JE (1993): Rapid diagnosis of enterovirus infections by magnetic bead extraction and polymerase chain reaction detection of enterovirus RNA in clinical specimens. Journal of Clinical Microbiology 31:31–38.

Munoz N, Bosch X, Kaldor JM (1988): Does human papillomavirus cause cervical cancer? The state of epidemiological evidence. British Journal of Cancer 57:1–5.

O'Banion MK, Sundberg JM, Rezka AA, Reichmann ME (1987): Cross-hybridization and relationships of various papillomavirus DNAs at different degrees of stringency. Intervirology 28:114–121.

Pakarian FB, Kaye JM, Cason J, Kell B, Jewers RJ, Raju KS, Best JM (1994): Cancer associated human papillomaviruses: Perinatal transmission and persistence. British Journal of Obstetrics and Gynaecology 101:514–517.

Saiki RA, Bugawan TL, Horn GT, Mullis KB, Erlich HA (1986): Analysis of enzymatically amplified β-globin and HLA DQ alpha DNA with allele specific oligonucleotide probes. Nature 324:163–166.

Schiffman MH, Bauer HM, Lorinccz AT, Manos MM, Bryne JC, Glass AG, Cadell DM, Howley PM (1991): Comparison of southern blot hybridization and polymerase chain reaction methods for the detection of human papillomavirus DNA. Journal of Clinical Microbiology 29:573–577.

Syrjanen KLJ (1989): Epidemiology of human papillomavirus infections and their associations with genital squamous cell cancer. APMIS 97:957–970.

Terry G, Ho L, Szarewski A, Cuzick J (1994): Semiautomated detection of human papillomavirus DNA of high and low oncogenic potential in cervical smears. Clinical Chemistry 40:1890–1892.

van den Brule AJC, Meijer CJLM, Bakels V, Kenemans P, Walboomers JMM (1990): Rapid detection of human papillomavirus in cervical scrapes by combined general primer mediated and type specific polymerase chain reaction. Journal of Clinical Microbiology 28:2739–2743.

zur Hausen H (1989): Papillomavirus in anogenital cancer as a model to understand the role of viruses in human cancer. Cancer Research 49:4677–4681.

zur Hausen H (1994): Molecular pathogenesis of cancer of the cervix and its causation by specific human papillomavirus types. Current Topics in Microbiology and Immunology 186:131–136.